WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, June 23, 2005

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L5	L1 same (Y245G or Y42R or W82R)	2
☐	L4	L2 same Y245G	1
☐	L3	L2 same (variant or mutant)	6
☐	L2	L1 same cellulolyticus	40
☐	L1	endoglucanase	1340

END OF SEARCH HISTORY

09/992504

=> d his

(FILE 'HOME' ENTERED AT 07:31:52 ON 23 JUN 2005)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 07:32:09 ON 23 JUN 2005

SEA (GLYCOSYL HYDROLASE) OR (ENDOGLUCANASE)

1	FILE ADISCTI
669	FILE AGRICOLA
15	FILE ANABSTR
13	FILE ANTE
1	FILE AQUALINE
49	FILE AQUASCI
353	FILE BIOBUSINESS
15	FILE BIOCOMMERCE
826	FILE BIOENG
2511	FILE BIOSIS
1072	FILE BIOTECHABS
1072	FILE BIOTECHDS
1198	FILE BIOTECHNO
894	FILE CABA
22	FILE CANCERLIT
3805	FILE CAPLUS
487	FILE CEABA-VTB
7	FILE CIN
38	FILE CONFSCI
2	FILE CROPB
17	FILE CROPU
4	FILE DDFB
3	FILE DDFU
2847	FILE DGENE
171	FILE DISSABS
4	FILE DRUGB
7	FILE DRUGU
22	FILE EMBAL
1304	FILE EMBASE
1209	FILE ESBIOBASE
41	FILE FEDRIP
2	FILE FOREGE
106	FILE FROSTI
703	FILE FSTA
2171	FILE GENBANK
2	FILE HEALSAFE
273	FILE IFIPAT
204	FILE JICST-EPLUS
4	FILE KOSMET
1366	FILE LIFESCI
4648	FILE MEDLINE
54	FILE NTIS
12	FILE OCEAN
1161	FILE PASCAL
37	FILE PCTGEN
1	FILE PHIN
15	FILE PROMT
1	FILE RDISCLOSURE
2818	FILE SCISEARCH
1681	FILE TOXCENTER
1216	FILE USPATFULL
97	FILE USPAT2

```
                1   FILE VETB
               19   FILE VETU
                3   FILE WATER
              251   FILE WPIDS
                3   FILE WPIFV
              251   FILE WPINDEX
L1               QUE (GLYCOSYL HYDROLASE) OR (ENDOGLUCANASE)
                 ---------

     FILE 'MEDLINE, CAPLUS, SCISEARCH, BIOSIS, TOXCENTER, LIFESCI, EMBASE,
     ESBIOBASE, BIOTECHNO, PASCAL, BIOTECHDS' ENTERED AT 07:34:26 ON 23 JUN
     2005
L2          22773 S L1
L3              2 S L2 AND (GLYCOSYL-STABILIZING)
L4              4 S L2 AND (INCREASED SPECIFIC ACTIVITY)
L5              1 DUP REM L4 (3 DUPLICATES REMOVED)
L6            983 S L2 AND (SPECIFIC ACTIVITY)
L7              0 S L6 AND (GLYCOSYL STABILI?)
L8              0 S L6 AND (POSITION 245)
L9             11 S L6 AND CELLULOLYTICUS
L10             7 DUP REM L9 (4 DUPLICATES REMOVED)
L11          2805 S L2 AND (MUTANT OR VARIANT)
L12            31 S L11 AND CELLULOLYTICUS
L13            12 DUP REM L12 (19 DUPLICATES REMOVED)
L14            12 S L2 AND (Y245G OR Y42R OR W82R)
L15             3 DUP REM L14 (9 DUPLICATES REMOVED)
L16             1 S L2 AND (TYR245GLY OR TYR42ARG OR TRP82ARG)

=>
```

L13 ANSWER 1 OF 12 Elsevier BIOBASE COPYRIGHT 2005 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2005130914 ESBIOBASE
TITLE: Catalytically enhanced endocellulase Cel5A from Acidothermus **cellulolyticus**
AUTHOR: Baker J.O.; McCarley J.R.; Lovett R.; Yu C.-H.; Adney W.S.; Rignall T.R.; Vinzant T.B.; Decker S.R.; Sakon J.; Himmel M.E.
CORPORATE SOURCE: J.O. Baker, National Bioenergy Center, National Renewable Energy Laboratory, 1617 Cole Boulevard, Golden, CO 80401, United States.
E-mail: john_baker@nrel.gov
SOURCE: Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology, (2005), 121-124/-(129-148), 40 reference(s)
CODEN: ABIBDL ISSN: 0273-2289
DOCUMENT TYPE: Journal; Conference Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English

AB When Tyr245 in endocellulase Cel5A from Acidothermus **cellulolyticus** was changed to Gly (Y245G) by designed mutation, the value of K.sub.i for inhibition of the enzyme by the product cellobiose was increased more than 1480%. This reduction in product inhibition enabled the **mutant** enzyme (used in conjunction with Trichoderma reesei cellobiohydrolase-I) to release soluble sugars from biomass cellulose at a rate as much as 40% greater than that achieved by the wild-type (WT) enzyme. The **mutant** was designed on the basis of the previously published crystal structure of the WT enzyme/substrate complex (at a resolution of 2.4 Å), which provided insights into the enzyme mechanism at the atomic level and identified Tyr245 as a key residue interacting with a leaving group. To determine the origin of the change in activity, the crystal structure of Y245G was solved at 2.4-Å resolution to an R-factor of 0.19 (R-free = 0.25). To obtain additional information on the enzyme-product interactions, density functional calculations were performed on representative fragments of the WT Cel5A and Y245G. The combined results indicate that the loss of the platform (Y245G) and of a hydrogen bond (from a conformational change in GLn247) reduces the binding energy between product and enzyme by several kilo calories per mole. Both kinetic and structural analyses thus relate the increased enzymatic activity to reduced product inhibition. Copyright .COPYRGT. 2005 by Humana Press Inc. All rights of any nature whatsoever reserved.

L13 ANSWER 2 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2005-01708 BIOTECHDS
TITLE: Novel **endoglucanase** MTE1 protein useful for processing cellulose containing fiber, deinking paper, improving filtrate ability of paper pulp or improving digestive ability of animal feed;
vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production and cellulose degradation
PATENT ASSIGNEE: MEIJI SEIKA KAISHA LTD
PATENT INFO: JP 2004313022 11 Nov 2004
APPLICATION INFO: JP 2003-107958 11 Apr 2003
PRIORITY INFO: JP 2003-107958 11 Apr 2003; JP 2003-107958 11 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2004-806980 [80]

AB DERWENT ABSTRACT:
NOVELTY - An **endoglucanase** protein (I) comprising a fully

defined sequence (S1) of 266 amino acids as given in the specification, (S1) in which one or more amino acids are substituted, deleted, added or inserted or an amino acid sequence that is 80% or more homologous with (S1), is new.

DETAILED DESCRIPTION - An **endoglucanase** protein (I) comprising a fully defined sequence (S1) of 266 amino acids as given in the specification, (S1) in which one or more amino acids are substituted, deleted, added or inserted or an amino acid sequence that is 80% or more homologous with (S1) or N-terminal of a sequence (S2) of Ala-Ala-Asp-Gly-Lys-Ser-Thr-Arg-Tyr-Trp-Asp-Cys-Cys-Lys-Pro-Ser. INDEPENDENT CLAIMS are also included for the following: (1) a polynucleotide (II) that encodes (I), comprising a fully defined sequence (S3) of 801 nucleotides as given in the specification, (S3) in which one or more nucleotides are substituted, deleted, added or inserted, a nucleotide sequence that is 80% or more homologous with (S3) or a nucleotide sequence that hybridizes under stringent conditions with (S3); (2) a recombinant vector (III) comprising (II); (3) a host cell (IV) transformed with (III); (4) producing (I), involves culturing (IV) and extracting (I) from (IV); (5) a cellulase preparation (V) comprising (I); (6) a granular form of stable detergent (VI) comprising (I) or (V), which does not have dustability property; and (7) a detergent composition (VII) comprising (I) or (V).

BIOTECHNOLOGY - Preferred Protein: (I) is obtained from a filamentous-fungi e.g., Myriococcum thermophilum. Preferred Polynucleotide: (II) comprises nucleotide sequence that is 90%, 95% or more homologous with (S3). Preferred Host Cell: (IV) is yeast (e.g., Saccharomyces cerevisiae, Hansenula or Pichia) or a filamentous fungi (e.g., Humicola insolens, Aspergillus niger, Trichoderma viridae, Fusarium oxysporum, Penicillium simplicissimum or Acremonium **cellulolyticus**).

USE - (I) or (V) is useful for processing cellulose containing fiber, decreasing fluff of cellulose containing fiber, improving exterior of cellulose containing fiber, processing colored cellulose-containing fiber, decreasing stiffness of cellulose containing fiber, deinking paper, improving filtrate ability of a paper pulp or improving digestive ability of animal feed. The cellulose containing fiber is further subjected to washing or rinsing processes (claimed).

ADVANTAGE - (I) enables to decrease fluff of cellulose containing fiber, improve exterior of cellulose containing fiber, decrease stiffness of cellulose containing fiber, improve filtrate ability of a paper pulp or improve digestive ability of animal feed.

EXAMPLE - To isolate **endoglucanase** protein, Myriococcum thermophilum FERGUS MBL2883 (FERM P-18841) strain was cultured in potato dextrose agar (PDA) culture medium at 25 degrees C. The cultured cells were recovered and were crushed. The culture supernatant was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), to purify **endoglucanase** protein. The obtained protein was sequenced. The protein comprised a fully defined sequence of 266 amino acids as given in the specification and N-terminal sequence of Ala-Ala-Asp-Gly-Lys-Ser-Thr-Arg-Tyr-Trp-Asp-Cys-Cys-Lys-Pro-Ser. (33 pages)

L13 ANSWER 3 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-11426 BIOTECHDS
TITLE: Novel thermal tolerant mannanase A polypeptide derived from Acidothermus **cellulolyticus**, useful for reducing hemicellulose in a starting material, for processing of food, and as bulking agents in food stuffs;
vector-mediated gene transfer and expression in host cell for recombinant enzyme production
AUTHOR: DING S; ADNEY W S; VINZANT T B; HIMMEL M E
PATENT ASSIGNEE: MIDWEST RES INST
PATENT INFO: WO 2003012110 13 Feb 2003

APPLICATION INFO: WO 2001-US23819 28 Jul 2001
PRIORITY INFO: WO 2001-23819 28 Jul 2001; WO 2001-23819 28 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-248182 [24]
AB DERWENT ABSTRACT:

NOVELTY - An isolated thermal tolerant mannanase A polypeptide (I) derived from Acidothermus **cellulolyticus**, comprising a sequence (S1) of 762 amino acids, and the fragments of (I) such as catalytic domain glycoside hydrolase (GH) 5, carbohydrate binding domain (CBD) type III, and CBD type II, having 411, 608, and 762 amino acids, respectively, is new. All sequences are given in the specification.

DETAILED DESCRIPTION - An isolated thermal tolerant mannanase A polypeptide (I) derived from Acidothermus **cellulolyticus**, comprising a sequence of 762 amino acids. The fragments of (I) such as catalytic domain, carbohydrate binding domain type III, and carbohydrate binding domain type II, have 411, 608, and 762 amino acids, respectively; or a sequence having at least 70% identity with the above sequences. All sequences are defined in the specification. INDEPENDENT CLAIMS are also included for: (1) a composition (II) comprising (I); (2) an industrial mixture suitable for degrading hemicellulose, comprising (I); (3) an isolated polynucleotide molecule (III) comprising a nucleic acid sequence having 90% sequence identity to a sequence encoding S1, or encoding a heterologous protein in frame with S1; (4) a mannanase substrate complex comprising (I) bound to hemicellulose; (5) a vector comprising (III); (6) a host cell genetically engineered to express (III); (7) a composition comprising (I) and a carrier; (8) an isolated antibody (IV) that specifically binds to (I); (9) production of (I); (10) a set of amplification primers (V) for amplification of a polynucleotide molecule encoding mannanase A, comprising two or more sequences having 9 or more contiguous nucleic acids derived from (III); and(11) a probe (VI) for hybridizing to a polynucleotide encoding mannanase A comprising a sequence of 9 or more contiguous nucleic acid derived from (III).

WIDER DISCLOSURE - Also disclosed are: (1) recombinant forms of (I); (2) **variants**, derivatives and fusion proteins of (I); and (3) reagents, compositions, and methods that are useful for analysis of ManA activity.

BIOTECHNOLOGY - Preparation: (I) is produced by incubating the above mentioned host cell (claimed). Preferred Mixture: The industrial mixture further comprises a detergent.

USE - (V) is useful for the detection of a mannanase A polynucleotide, by amplifying a nucleic acid sequence with (V), and correlating the amplified nucleic acid sequence with detected polynucleotide encoding mannanase A. (I) is useful for assessing the carbohydrate degradation activity of mannanase A, by analyzing a carbohydrate degradation in the presence of mannanase A and a carbohydrate degradation in the absence of mannanase A on a substrate, and comparing the carbohydrate degradation in the presence of mannanase A with the carbohydrate degradation in the absence of mannanase A. The method is also carried out in the presence of a desired agent. (I) is also useful for reducing hemicellulose in a starting material (claimed), to simpler carbohydrate units, ultimately to sugars which are useful in the food, feed, paper pulp, and biofuels industries. (I) is useful for the processing of food and in food stuffs as bulking agents. Fragments of (I) are useful to generate specific anti-ManA antibodies. (I) is also useful to raise polyclonal and monoclonal antibodies that are useful in purifying ManA, or detecting ManA polypeptide expression, and as well as reagent tool for characterizing the molecular actions of ManA polypeptides. (II) is useful for removal of hemicellulose containing stains within fabrics and in pulp and paper industry to address conditions associated with hemicellulose contamination of the cellulose fraction. (II) is also useful to produce oligosaccharide bulking agents and stabilizers from hemicellulose for use in the food and feed industry.

EXAMPLE - Molecular cloning of mannanase A (ManA) was as follows. Genomic DNA was isolated from Acidothermus **cellulolyticus** and purified. The purified genomic DNA was then digested and separated on agarose gels. DNA fragments in the range of 9-20 kilobase pairs were isolated from the gels. This purified genomic DNA was ligated into the BamH1 acceptor site of purified EMBL3 lambda phage arms. Phage DNA was packaged and plated with Escherichia coli LE392 in top agar which contained the soluble cellulose analog, and carboxymethylcellulose. The plates were then incubated overnight to allow transfection, bacterial growth, and plaque formation. Plates were stained followed by destaining. lambda plaques harboring **endoglucanase** clones showed up as unstained plaques. lambda clones which screened positive were purified. Individual phage isolates were named SL-1, SL-2, SL-3, and SL-4. Subsequent subcloning efforts employed the SL-3 clone which contained 14.2 kb fragment of A. **cellulolyticus** genomic DNA. Template DNA was constructed using a 9 kb BamH1 fragment obtained from 14.2 kb lambda clone SL3 prepared from A. **cellulolyticus** genomic DNA. The 9-kb BamHI fragment from SL3 was subcloned into pDR540 to generate a plasmid NREL501. NREL501 was first sequenced, then subcloned into pUC19, and transformed into E. coli XL1-blue for the production of template DNA for sequencing. Each subclone was sequenced, and the sequencing data from primer walking and subclones were assembled together to verify that all SL3 regions had been sequenced. An open reading frame (ORF) was found in the 9-kb BamHI fragment, C-terminal of E1, and then termed as ManA. An ORF of 2289 bp defined in the specification and its deduced amino acid sequences having 762 amino acids were obtained. The amino acid sequence predicted was determined to have significant homology to known mannanases.(46 pages)

L13 ANSWER 4 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-11180 BIOTECHDS
TITLE: Novel thermal tolerant GuxA polypeptide derived from Acidothermus **cellulolyticus**, useful for reducing cellulose in a starting material, and for the conversion of biomass to biofuels and biofuel additives; vector-mediated recombinant protein gene transfer and expression in host cell for use in carbohydrate degradation, cellulose degradation, fuel, surfactant, paper mill and monoclonal antibody preparation
AUTHOR: DING S; ADNEY W S; VINZANT T B; HIMMEL M E; DECKER S R
PATENT ASSIGNEE: MIDWEST RES INST
PATENT INFO: WO 2003012109 13 Feb 2003
APPLICATION INFO: WO 2001-US23817 28 Jul 2001
PRIORITY INFO: WO 2001-23817 28 Jul 2001; WO 2001-23817 28 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-239526 [23]
AB DERWENT ABSTRACT:

NOVELTY - An isolated thermal tolerant GuxA polypeptide (I) derived from Acidothermus **cellulolyticus**, having a sequence (S1) of 1228 amino acids (aa). Fragments of (I) e.g., first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (CBD) type III, and CBD type II, having 423, 231, 150, and 101 aas, respectively, is new. All sequences are fully disclosed in the specification.

DETAILED DESCRIPTION - An isolated thermal tolerant GuxA polypeptide (I) derived from Acidothermus **cellulolyticus**, having a sequence (S1) of 1228 amino acids (aa). Fragments of (I) e.g., first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (CBD) type III, and CBD type II, having 423, 231, 150, and 101 aas, respectively. (I) has a sequence (S1) of 1228 aas. The fragments of (I) such as first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (CBD) type III, and CBD type

II, having 423, 231, 150, and 101 aas, respectively; or a sequence having at least 70% identity with the above sequences. INDEPENDENT CLAIMS are also included for the following; (1) composition (II) comprising (I); (2) an industrial mixture suitable for degrading cellulose, comprising (I); (3) fusion protein (III) comprising (I) and a heterologous peptide; (4) cellulase-substrate complex comprising (I) bound to cellulose; (5) vector comprising the polynucleotide that encodes (I); (6) host cell genetically engineered to express (I); (7) composition comprising (I) and a carrier; (8) isolated antibody (IV) that specifically binds to (I); (9) production of (I); (10) set of amplification primers (V) for amplification of a polynucleotide molecule encoding GuxA, comprising two or more sequences having 9 or more contiguous nucleic acids derived from the polynucleotide molecule; and (11) probe (VI) for hybridizing to a polynucleotide encoding GuxA comprising a sequence of 9 or more contiguous nucleic acid derived from the polynucleotide molecule.

WIDER DISCLOSURE - Also disclosed as new are the following: (1) recombinant forms of (I); (2) **variants**, and derivatives of (I); (3) reagents, compositions, and methods that are useful for analysis of GuxA activity; and (4) a polynucleotide encoding (I).

BIOTECHNOLOGY - Preparation: (I) is produced by incubating the above mentioned host cell (claimed). Preferred Mixture: The industrial mixture further comprises a detergent. Preferred Fusion Protein: The heterologous peptide is a leucine zipper.

USE - The set of primers (V) are useful for the detection of a polynucleotide encoding GuxA, by amplifying a nucleic acid sequence with (V), and correlating the amplified nucleic acid sequence with detected polynucleotide encoding GuxA. (I) is useful for assessing the carbohydrate degradation activity of Gux A, by analyzing a carbohydrate degradation in the presence of GuxA and a carbohydrate degradation in the absence of GuxA on a substrate, and comparing the carbohydrate degradation in the presence of GuxA with the carbohydrate degradation in the absence of GuxA. The method is also carried out in the presence of desired agent. (I) is useful for reducing cellulose in a starting material such as agricultural biomass (all claimed), to sugars which is useful in biofuel production. (I) is useful in the conversion of biomass to biofuels and biofuel additives, in detergents pulp and paper processing, food and feed processing, and in textile process. Fragments of (I) are useful to generate specific anti-GuxA antibodies. (I) is also useful to raise polyclonal and monoclonal antibodies that are useful in purifying GuxA, or detecting GuxA polypeptide expression, and as well as reagent tool for characterizing the molecular actions of GuxA polypeptides.

EXAMPLE - Molecular cloning of GuxA was as follows: Genomic DNA was isolated from Acidothermus **cellulolyticus** and purified. The purified genomic DNA was then digested and separated on agarose gels. DNA fragments in the range of 9-20 kilobase pairs were isolated from the gels. This purified genomic DNA was ligated into the BamH1 acceptor site of purified EMBL3 lambda phage arms. Phage DNA was packaged and plated with Escherichia coli LE392 in top agar which contained the soluble cellulose analog, and carboxymethylcellulose. The plates were then incubated overnight to allow transfection, bacterial growth, and plaque formation. Plates were stained followed by destaining. lambda plaques harboring **endoglucanase** clones showed up as unstained plaques. lambda clones which screened positive were purified. Individual phage isolates were named SL-1, SL-2, SL-3, and SL-4. Subsequent subcloning efforts employed the SL-3 clone which contained 14.2 kb fragment of A. **cellulolyticus** genomic DNA. Template DNA was constructed using a 9 kb BamH1 fragment obtained from 14.2 kb lambda clone SL3 prepared from A.**cellulolyticus** genomic DNA. The 9-kb BamHI fragment from SL3 was subcloned into pDR540 to generate a plasmid NREL501. NREL501 was first sequenced, then subcloned into pUC19, and transformed into E.coli XL1-blue for the production of template DNA for sequencing. Each subclone was sequenced, and the sequencing data from primer walking and subclones

were assembled together to verify that all SL3 regions had been sequenced. An open reading frames (ORF) was found in the 9-kb BamHI fragment, C-terminal of E1, and then termed as GuxA. An ORF of 3687 bp defined in the specification and its deduced aa sequences having 1228 aas were obtained. The aa sequence predicted was determined to have significant homology to known cellulases. (47 pages)

L13 ANSWER 5 OF 12 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002277395 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12018266
TITLE: Effect of single active-site cleft mutation on product specificity in a thermostable bacterial cellulase.
AUTHOR: Rignall Tauna R; Baker John O; McCarter Suzanne L; Adney William S; Vinzant Todd B; Decker Stephen R; Himmel Michael E
CORPORATE SOURCE: Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401, USA.
SOURCE: Applied biochemistry and biotechnology, (2002 Spring) 98-100 383-94.
Journal code: 8208561. ISSN: 0273-2289.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20020522
Last Updated on STN: 20021211
Entered Medline: 20021106

AB Mutation of a single active-site cleft tyrosyl residue to a glycyl residue significantly changes the mixture of products released from phosphoric acid-swollen cellulose (PSC) by EIcd, the catalytic domain of the **endoglucanase**-I from Acidothermus **cellulolyticus**. The percentage of glucose in the product stream is almost 40% greater for the Y245G **mutant** (and for an additional double **mutant**, Y245G/Q204A) than for the wild type enzyme. Comparisons of results for digestion PSC and of pretreated yellow poplar suggest that the observed shifts in product specificity are connected to the hydrolysis of a more easily digestible fraction of both substrates. A model is presented that relates the changes in product specificity to a mutation-driven shift in indexing of the polymeric substrate along the extended binding-site cleft.

L13 ANSWER 6 OF 12 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002277384 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12018255
TITLE: Exploration of cellulose surface-binding properties of acidothermus **cellulolyticus** Cel5A by site-specific mutagenesis.
AUTHOR: McCarter Suzanne L; Adney William S; Vinzant Todd B; Jennings Edward; Eddy Fannie Posey; Decker Stephen R; Baker John O; Sakon Joshua; Himmel Michael E
CORPORATE SOURCE: Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401, USA.
SOURCE: Applied biochemistry and biotechnology, (2002 Spring) 98-100 273-87.
Journal code: 8208561. ISSN: 0273-2289.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20020522

Last Updated on STN: 20021211
Entered Medline: 20021106

AB Understanding the interactions between cellulases and cellulosic substrates is critical to the development of an efficient artificial cellulase system for conversion of biomass to sugars. We directed specific mutations to the interactive surface of the Acidothermus **cellulolyticus** E1 **endoglucanase** catalytic domain. The cellulose-binding domain is not translated in these **mutants**. Amino acid mutations were designed either to change the surface charge of the protein or to modify the potential for hydrogen bonding with cellulose. The relationship between cellulase-to-cellulose (Avicel PH101) binding and hydrolysis activity was determined for various groupings of mutations. While a significant increase in hydrolysis activity was not observed, certain clusters of residues did significantly alter substrate binding and some interesting correlations emerged. In the future, these observations may be used to aid the design of **endoglucanases** with improved performance on pretreated biomass.

L13 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:824394 CAPLUS
DOCUMENT NUMBER: 134:2062
TITLE: Acidothermus **cellulolyticus** E1 **endoglucanase variants** Y245G, Y82R and W42R with increased catalytic activity
INVENTOR(S): Himmel, Michael E.; Adney, William S.; Baker, John O.; Vinzant, Todd B.; Thomas, Steven R.; Sakon, Joshua; Decker, Stephen R.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2000070031	A1	20001123	WO 2000-US13971		20000519
WO 2000070031	C2	20020704			
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
CA 2372594	AA	20001123	CA 2000-2372594		20000519
AU 2000052791	A5	20001205	AU 2000-52791		20000519
EP 1179051	A1	20020213	EP 2000-937647		20000519
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO					
US 2003054535	A1	20030320	US 2001-997504		20011119
PRIORITY APPLN. INFO.:			US 1999-134925P	P	19990519
			WO 2000-US13971	W	20000519

AB The invention provides a method for making a **glycosyl hydrolase** characterized by an increase in catalytic activity on an insol. substrate. An active site associated glycosyl-stabilizing amino acid of the hydrolase is thus replaced with an amino acid, the replacing amino acid not strongly binding a disaccharide product in the active site. The method for making a **glycosyl hydrolase** characterized by an increase in catalytic activity on a soluble substrate comprises

replacing a hydrophobic substrate binding amino acid of the hydrolase with a pos. charged amino acid. The invention specifically provides Acidothermus **cellulolyticus** E1 **endoglucanase** **variants**, comprising Y42R, W82R, or Y245G, and the DNA sequences encoding the enzymes. Ki values for inhibition of hydrolysis of 4-β-D-cellobioside by native and Y245G **mutant** E1 indicate that the **mutant** catalytic domain binds cellobiose 15-fold less tightly than does the native enzyme, i.e., an increase in Ki from 2 to 30 mM cellobiose and a decrease in apparent binding energy of 1.7 kcal/mol.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:1078863 CAPLUS
DOCUMENT NUMBER: 142:351169
TITLE: Sequences of Acidothermus **cellulolyticus** E1 **endoglucanase** cellulose binding domain
INVENTOR(S): Himmel, Michael E.; Thomas, Steven R.; Laymon, Robert A.; Adney, William S.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: Can. Pat. Appl., 86 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2226898	AA	19990925	CA 1998-2226898	19980325
PRIORITY APPLN. INFO.:			CA 1998-2226898	19980325

AB The Acidothermus **cellulolyticus** E1 **endoglucanase**, domains thereof, fusion proteins containing such domains, and **variants** thereof are described. Nucleic acids encoding such proteins or polypeptides are also described. The cellulose binding domain of the E1 **endoglucanase** is useful in labeling or modifying a cellulose or other polysaccharide surface, and in purifying or immobilizing a binding domain fusion protein to cellulose or other polysaccharide.

L13 ANSWER 9 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1999-00710 BIOTECHDS
TITLE: Engineering cellulase systems: enzyme modifications for improved function;
recombinant thermostable **endoglucanase** production, purification and characterization by Escherichia coli (conference abstract)
AUTHOR: Himmel M E; Adney W S; Baker J O; Decker S R; Vinzant T B; Nieves R A; Godbole S; Thomas S R
CORPORATE SOURCE: Nat.Renewable-Energy-Lab.Colorado
LOCATION: National Renewable Energy Laboratory, Golden, CO, USA.
SOURCE: Abstr.Gen.Meet.Am.Soc.Microbiol.; (1998) 98 Meet., 28
CODEN: 0005P
ISSN: 0067-2777
98th General Meeting of the American Society for Microbiology, Atlanta, GA, USA, 17-21 May, 1998.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In general, cellulase (EC-3.2.1.4) systems of interest to industry would be more cost effective if they displayed higher specific activities. Acidothermus **cellulolyticus** EI is a thermotolerant **endoglucanase**, which was isolated from a hot spring bacterium that demonstrated very high synergism with fungal cellobiohydrolase (EC-3.2.1.91). A recent 1.8 Angstrom crystallographic structure was the

basis of the current strategy to improve the EI catalytic domain (cd) by polymerase chain reaction mutation. Modifications were targeted towards areas of the enzyme that could improve its saccharification of biomass. Polymerase chain reaction was used to generate 18 **mutant** EI coding sequences and following verification of the mutation sites by polymerase chain reaction DNA sequencing, microtiter plate assays were employed to determine that 13 of these **mutants** yielded active enzymes. Transformed Escherichia coli, which expressed this enzyme, was then grown and each active **mutant** enzyme was purified to homogeneity using a novel 3-step column chromatographic method. (0 ref)

L13 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 1996:262842 CAPLUS
DOCUMENT NUMBER: 124:309585
TITLE: Gene coding for the E1 **endoglucanase** from Acidothermus **cellulolyticus**
INVENTOR(S): Thomas, Steven R.; Laymon, Robert A.; Himmel, Michael E.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: PCT Int. Appl., 33 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9602551	A1	19960201	WO 1995-US8868	19950714
W: AU, BR, CA, CN, DE, ES, GB, JP, KP, KR, NZ, SE				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5536655	A	19960716	US 1994-276213	19940715
AU 9530985	A1	19960216	AU 1995-30985	19950714
AU 682125	B2	19970918		
EP 771321	A1	19970507	EP 1995-926694	19950714
R: DE, ES, FR, GB				

PRIORITY APPLN. INFO.:

US 1994-276213	A	19940715
US 1989-412434	A2	19890926
US 1992-826089	A2	19920127
US 1993-125115	A2	19930921
WO 1995-US8868	W	19950714

AB The gene encoding Acidothermus **cellulolyticus** E1 **endoglucanase** was cloned and expressed in heterologous microorganisms by standard recombinant DNA techniques. The 3004-bp fragment of DNA contains a coding portion of 1686 bp corresponding to a deduced protein sequence of 562 amino acids and containing a 41-residue signal moiety. The gene architecture is similar to that of cellulase genes isolated from other actinomycete bacteria. New modified E1 **endoglucanase** enzymes are produced along with **variants** of the gene and enzyme. The E1 **endoglucanase** is useful for hydrolyzing cellulose to sugars for simultaneous or later fermentation into alc. Recombinant production (e.g., in Escherichia coli or Saccharomyces lividans) result in much improved rate of of enzyme production, thereby lowering the cost of cellulase and the production of alc. using cellulosic materials as substrate.

L13 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 1996:467404 CAPLUS
DOCUMENT NUMBER: 125:107081
TITLE: Gene encoding the E1 **endoglucanase** from Acidothermus **cellulolyticus**
INVENTOR(S): Thomas, Steven R.; Laymon, Robert A.; Himmel, Michael E.
PATENT ASSIGNEE(S): Midwest Research Institute, USA

SOURCE: U.S., 21 pp., Cont.-in-part of U.S. 5, 366, 884.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5536655	A	19960716	US 1994-276213	19940715
US 5110735	A	19920505	US 1989-412434	19890926
EP 885955	A2	19981223	EP 1998-108104	19900827
EP 885955	A3	19990407		
R: DE, FR, GB				
US 5275944	A	19940104	US 1992-826089	19920127
US 5366884	A	19941122	US 1993-125115	19930921
CA 2194478	AA	19960201	CA 1995-2194478	19950714
WO 9602551	A1	19960201	WO 1995-US8868	19950714
W: AU, BR, CA, CN, DE, ES, GB, JP, KP, KR, NZ, SE				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9530985	A1	19960216	AU 1995-30985	19950714
AU 682125	B2	19970918		
EP 771321	A1	19970507	EP 1995-926694	19950714
R: DE, ES, FR, GB				
US 5712142	A	19980127	US 1996-604913	19960222

PRIORITY APPLN. INFO.:

US 1989-412434	A2	19890926
US 1992-826089	A2	19920127
US 1993-125115	A2	19930921
EP 1990-914450	A3	19900827
US 1994-276213	A	19940715
WO 1995-US8868	W	19950714

AB The gene encoding Acidothermus **cellulolyticus** E1 **endoglucanase** is cloned, sequenced, and expressed in heterologous microorganisms by standard recombinant DNA techniques. The 3004-bp sequence encodes a 562-amino acid precursor enzyme containing a 41-residue signal sequence which is cleaved to yield the active E1 **endoglucanase** enzyme. New modified E1 **endoglucanase** enzymes can be produced by standard techniques of mutagenesis and mixed domain construction. The E1 **endoglucanase** is useful for hydrolyzing cellulose to sugars for simultaneous or later fermentation into alc.

L13 ANSWER 12 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1987-08686 BIOTECHDS
TITLE: Bacterium produces thermostable cellulase; Acidothermus **cellulolyticus** enzyme characterization
AUTHOR: Seltzer R
LOCATION: (Pub. Address) American Chemical Society, 1155 Sixteenth Street NW, Washington D.C. 20036, USA.
SOURCE: Chem.Eng.News; (1987) 65, 18, 23-24
CODEN: CENEAR
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Scientists at Solar Energy Research Institute (SERI) in Golden, Colorado, have found a cellulase (EC-3.2.1.4) showing activity and stability at temperatures far higher than for any previously known cellulase. The enzyme is produced by the newly discovered Acidothermus **cellulolyticus**, and is potentially useful for economic fuel ethanol production from cellulosic biomass. The thermostable cellulase is also of interest in the food industry. A. **cellulolyticus** is moderately thermophilic and shows optimal cell growth at pH 5 and 55 deg. It is aerobic, utilizes a variety of substrates, and secretes a complex of cellulase enzymes. Crude culture broths from the bacterium show

optimal temperatures of 75 deg for total cellulase activity and 83 deg for **endoglucanase** activity. At 95 deg, 38% of total activity and 60% of **endoglucanase** activity remain. Work is in progress regarding the isolation and characterization of the A. **cellulolyticus** cellulase enzymes, and the ultimate goal is the use of recombinant DNA technology to produce cellulase hyperproducing **mutants**. (0 ref)